

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q96905

Hitoshi AOKI, et al.

Appl. No.: 10/593,465

Group Art Unit: 1614

Confirmation No.: 2689

Examiner: Kevin E WEDDINGTON

Filed: September 19, 2006

For: INHIBITOR OF BLOOD GLUCOSE LEVEL ELEVATION AND INHIBITOR OF AGE
GENERATION COMPRISING ACEROLA LEAF EXTRACT AND FOOD PRODUCT
COMPRISING EITHER THEREOF

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Takayuki HANAMURA, hereby declare and state:

THAT I am a citizen of Japan;

THAT I have received the degree of master in 1998 from Tokyo Institute of Technology;

THAT I was employed by Nichirei Corporation in 1998, and transferred to and have been
employed by Nichirei Foods, Inc., where I have engaged in research and development relating to
functionality of food materials;

THAT I am familiar with the Office Action dated December 3, 2010;

THAT I am aware that Claims 9, 11, 18 and 19 have been rejected under 35 U.S.C. §
103(a) as allegedly being unpatentable over Leltz et al. (U.S. Patent No. 4,877,627); and

I submit the present Declaration in support of the patentability of the invention described
in the above-identified application over Leltz. My Declaration provides comparative HPLC data

showing that acerola leaf extracts and acerola fruit extracts contain different types of polyphenols.

Experiment

Standard High-Performance Liquid Chromatography (HPLC) was carried out on an acerola leaf extract and an acerola fruit extract. Leaf extract was prepared as follows. Acerola leaves were homogenized, and 3 x volume of distilled hot water was added thereto, followed by 1-hour extraction. This procedure was carried out twice, and the extract was centrifuged, filtered, lyophilized, and then diluted in distilled water again. The resulting solution was applied to the C18 cartridge columns (Sep-Pak Vac 35cc C18 cartridge columns, Waters), washed with distilled water, and eluted with a 0.2% TFA/methanol solution. The elution fraction was evaporated to dryness to obtain a purified extract. Fruit extract was prepared in a manner similar to "a purified extract" of example 1 in the present specification. The HPLC conditions were as follows.

Fig. 1: Acerola leaf extract

Column: Waters XTerra Phenyl (4.6 × 150 mm)
Temperature: 40°C
Flow rate: 0.8 ml/min
Mobile phase: Solvent A – 13% CH₃CN (with 0.1% THF); Solvent B – 100% CH₃CN (with 0.1% THF)
Detection wavelength: 350 nm

Fig. 2: Acerola fruit extract

Column: Develosil RPAQUAS C30 (Nomura Chemical) (10 × 250 mm)
Temperature: 40°C
Flow rate: 2.3 ml/min
Mobile phase: Solvent A – 20% CH₃CN (with 0.1% THF); Solvent B – Methanol
Gradient: 0-35 minutes A 100%
35-40 minutes B 100%
40-50 minutes A 100%

Detection wavelength: 280 nm

Results

The C18-adsorbed fraction of acerola leaf extract, extracted with hot water, shows that the acerola leaf polyphenol is mainly composed of kaempferol. See Fig. 1.

Acerola leaf extract

(A) C18-adsorbed fraction of acerola leaf extract extracted with hot water



(B) Hydrolysate

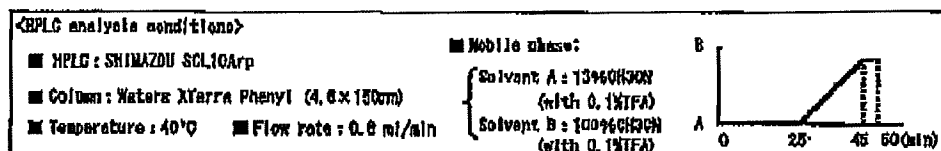
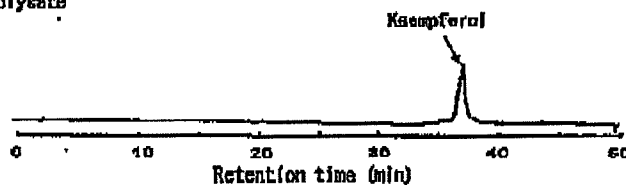
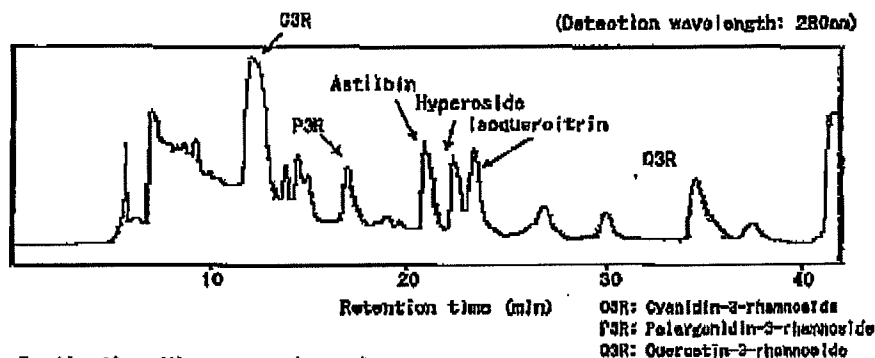


FIGURE 1

The C18-adsorbed fraction of acerola fruit extract, extracted with water, shows that the acerola fruit polyphenol is mainly composed of cyanidin-3-rhamnoside (C3R) and the amount of kaempferol is very small. See Fig. 2.

Acerola fruit extract

C18-adsorbed fraction of mature acerola fruit extract extracted with water



Fractionation with a reverse phase column

Column:	Develco RP400AS C30 Column (Omura Chemical) (10 x 250 mm)
Column oven temp.:	40°C
Mobile phase A:	20% Acetonitrile (0.1% TFA)
Mobile phase B:	Methanol
Gradient:	0-35 minutes A 100% 35-40 minutes B 100% 40-60 minutes A 100%
Flow rate:	2.3 ml/min

FIGURE 2

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 2011/3/3

Takayuki Hanamura
Takayuki HANAMURA